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Bordukova, M. V., New Developments in the control of phytopthora. Sad i Ogorod, No. 9, p. 62-65, Sept. 1947. 80 Sa13

There are no radical means for controlling phytophthora to date. The Scientific Research Institute of Potato Industry is engaged not only in verifying (checking) all known methods of controlling phytophthora, but in finding a new vegetative fungicide.

There is no doubt that such a vegetative fungicide exists. Numerous observations convince us in this supposition in noting the different degrees of contamination of potatoes by this disease. Plants of the same variety and age, grown in practically identical conditions with regard to relief of location and the physical structure of the soil, given the same amount of fertilizers, same moisture and density of planting, etc., are affected in different degrees in a year of severe outbreak of phytophthora. The foliage rots completely in the course of several days in the majority of plants, but on the same field, against the background of destroyed potatoes, it is possible to find altogether healthy specimens.

The hi

The history of this problem is extremel scant, although convincing.

We know on the basis of literary data that the population of the American Andes improved the keeping quality of potatoes by using a grass called "munia", smelling like mint, for layers in storing potato crops.

In the belief that the presence of some poisonous grasses interfered with the development of phytophthora, we tested, in 1941, such plants as "durman" [Datura stramonium], "belena", henbane [Hyoscyamus niger], "smut", gout weed [Aegopodium podograria], etc. The war interrupted our work in this direction. But even those few experiments established that plants which are poisonous to man and animals are far from poisonous to phytophthora.

The matter evidently lies not in poisonous plants, but in the phytoncides they possess and concerning which Tokin in his work "Vegetative bacteriocytes" writes: "Science does not know as yet what phytonicides are. They possibly are chemical substances of complex structure or are "rays" of unknown origin." Fungi react differently in the presence of various phytonicides. We tested about 130 plants of different families and made close to 150 experiments in order to establish the most active phytonicides and their reaction upon the development of phytophthora. Experiments on testing plants were made in special grass vessels-exicators?. At the bottom of the vessel were placed crushed leaves of the plant under test. Above them on glass bars, were placed artificially infected cut potato slices or entire tubers. The vessel was covered with a glass top.

In the very first series of tests seed garlic [Alliaria DC - Erysimum Alliaria Scop] revealed drastically its interference with the development of phytophthora. If on control tubers (i.e. infected tubers, kept away from the influence of garlic), the disease appeared on the 5-6th day, on tubers exposed to phytonicides on crushed garlic, there were no signs of infection even on the 12 day. Numerous tests conducted in the laboratory with artificially phytophthora infected potatoes invariably gave positive results.

The effect of these experiments is better understood if the direct action of phytoncides of garlic upon the fungus is examined. Usually the zoospores of the fungus are capable of floating freely in a drop of water with the aid of flagella, for as long as 20-30 minutes. But as soon as a particle of crushed garlic is introduced into the drop filled with active zoosporas, the movement of the latter stops within 20-50 seconds.

If a small quantity of garlic is placed close to the drop filled with zoospores (i.e. when only the smell of garlic affects the zoospores), they become immobile after one and a half, two minutes

The immobility of zoospores nevertheless does not significy the loss of their viability; our subsequent experiments established the duration of the action of phytoncides og arlic. It appeared that if one drop of garlic juice is introduced into 100 cu. cm. of water filled with zoospores, the latter lose their capacity for growth entirely in four hours. Prior to that they remain in a sort of paralized condition.

Of equal importance is the knowledge of how deep the garlic phytoncide penetrates into the tissue of the tuber. Is it capable to prevent infection in the event that the fungus already penetrated the tuber? It is to be noted that the penetration of the fungus into the tissue of the tuber lasts from 5 to 6 hours.

The experiment with ten infected tubers established that garlic phytonicides prevent the infestation of tubers, the tissue of which the fungus has already penetrated.

In all variants of this test the influence of garlic was observed for 12 days. Having convinced ourselves that garlic phytonicde stops the development of the fungus phytophthora, we started to prepare an experiment on 100 kg of potatoes. Finely crushed garlic was strewn at the botton of a box where potatoes were later stored. Next was placed a layer of artificially infested tubers (two tubers thick), then garlic, alternating again with potatoes and garlic. The top layer was garlic. Artificially infected tubers of potatoes, not exposed to garlic, were used as control.

All tubers were kept under conditions favoring the development of the disease. Moisture of 100 percent and the temperature of 20-21° C. were maintained in the box where potatoes were stored. The tubers were lightly covered with paper to prevent suffocation.

Seven days later control tubers were infested 100 percent; of tubers under test 84.8 percent had remained completely healthy and 15.2 percent were infested with phytotophthora.

To preserve almost 100 kg of potatoes one head of garlic (ab. 100g) had sufficed. Garlic rots eventually but this does not affect the tubers. Not all varieties of garlic react similarly, however, Some possess a yet unknown, disinfectant substance in large amount, others possess smaller amounts. The study of varieties of garlic will be continued.

It follows that fall storing of potatoes with crushed (pulverized garlic will greatly decrease losses in yield. Actually, the tubers are destroyed by phytophthora in the first three weeks of storing when the relative moisture of air in storing is high, the freshly dug potatoes are still wet and carry on their suface a mass of fruit... of the fungus, and the temperature in the quarters is higher than the required 1 - 2° C.

In studying phytonicides of numerous plants, we noted extremely interesting factors. If garlic had proved to be a controller or disinfectant of phytophthora, there are, evidently, other plants which contribute to the development and spread of the disease. Among similar stimulants are "lebeda", goosefoot? [Chenopodium], "ptichia grechishka" [Polygonaceae, Polygonum avium], "grechishka v"unkovaia" [P. convulvus L.]; "osot polevoi" [Sonchus arvensis L.]; "khvoshch polevoi" [Equisetum arvense L.] "Shchavelek maly" [Rumex L.] (small); "schavelek konskii" [R. confertus Willd], and many others.

These weeds are pests of potato plants not only because they deprive the latter of water and interfere with their nutrition, but also because they contribute to the development of phytophthora.

Moreover, it seems relevant to note the type of plants gorwing close to potatoes. Sunflowers, tomatores, apply, cherry trees, raspberries, squash, cucumbers are, for instance, clearly contributing to the development of the disease whily sugar beets, carrots, lettuce, dill, onions, parsley, cabbage and others hinder its development. It was established that potatoes grown on a plot surrounded by birch trees rot faster than those on plots bordered by pines.

We obtained data with regard to experiments on common rowan [Sorbusaucuparia] The Phytoncide of this plant proved stronger and more resistant even than garlic phytonicide.

At present effective dosages of phytonicides of Sorbus and the techniques for their application are being determined.

Golubinskii, I. N., Effect of onion phytoncides on the Germination of pollen grains. Priroda 38(3): 67-68 Mar. 1949. 410 P933

The first experiment in germination of the pollen in the Petri cups over the circles of onion bulbs which were cut across gave much unexpected results that they immediately attracted our attention. Really, the pollen of a series of plants sown upon freshly cut onion refused to sprout, while simultaneously it germinated well in control cups. Repetitive attempts of germinating the pollens and including into the experiments the pollens of new varieties of plants gave the same results.

During this experiment it has been determined that the presence of the pollen sown upon the nourishing parts of the cut onion not only checks the sprouting pollen grains, but kills them entirely, since the transfer of the pollen into fresh air, after having been five minutes in the atmosphere of phytoncides of onions did not save the pollen grains and they lost their ability to sprout forever.

It is characteristic, nevertheless, that the phytoncides of onion kill the pollen only after its sowing upon the nourishing parts for sprouting. The Phytoncides do not effect the dry pollen (at least during the effect of 24 hours with triple onion shift). The pollen of any of plants under experiment (about 15 varieties) after having been in dry conditions during twenty four hours in an atmosphere saturated by the phytoncides of onion and sown later for sprouting sprouted normally.

The mortal effect upon the sprouting pollen is indicated only by a freshly cut onion. When we sow the pollen upon an onion which has been cut half an hour ago they sprout normally.

Whole onion bulbs which were not cleansed from their external scale manifest a peculiar effect upon the growing pollen. A few small bulbs were placed into a cylinder, about 150 cm in size. In order to create in the cyliner a humid atmosphere, some water has been poured into the bottom. The cylinder has been covered by a glass plate into whose anterior some drops of the nourishing medium with the sown pollen were introduced. The task of this experiment was the study of the influence of whole, uncleaned bulbs upon the sprouting of pollen grains. It has been clarified that in this case, although the pollen grew somewhat normally, the thickness of the tubiflorae was inferior to the control one. Besides, in this case the medium and the tubiflorae somehow conserved, remaining unchanged up to six-eight days (under the temperature of 80 percent). It is quite characteristic that niehter in this case, nor with the sowing over the cut onion was it possible to observe fungi mycelia which were developing strongly in sugar solutions which serve as nourishing substrate for the sprouting of pollen grains.

Especially powerful effect of phytoncides upon the sprouting pollen tubiflorae is derived from the onion juice, whose minimal mixture hampers their sprouting.

The testing of the phytoncide effect of other plant varieties has confirmed the data of Prof. Tokin and of his assistants. Like other experiments with microorganisms, the phytoncides of other plants affect the pollen less than the onion phytoncides.

Kramarenko, L. R., [Bactericidal properties of cell sap as one of the factors conditioning varietal resistance of cotton to gummosis] Vsesoiuzn. Akad. Sel'skokhoz. Nauk im. V. I. Lenina. Dok. 14(2):36-40. 1949. 20 Akl

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[This is a longer paper dealing not with phytonicides but with the cell sap as a factor in varietal immunity of cotton to gummosis. The following conclusions are stated at the end of the article.]

Conclusions

1. One of the factors enabling the conditioning of varietal resistance of cotton to gummosis, is the bactericidal property of its cell sap, which is manifest in the bacteriostatic effect on cells of B. malvacearum.

2. There is a direct dependence between the bactericidal property of cotton's cell sap and the susceptibility of varieties to gummosis.

3. The degree of bactericidal property of cotton's cell sap increases with the plant's age. The bactericidal property of cell sap is lowest during the cotyledon phase, highest -during that of budding.

Lipetskaia, A. D., [Testing of phytoncides in the control of vascular bacteriosis of cabbage]. Sad i Ogorod 1950:51-52. Jan. 1950.

In 1928, Prof. B. P. Tokin discovered among various higher plants the presence of substances capable of destroying disease-originating micro-organisms; he called these substance phytoncides.

We decided to test phytoncides for control of diseases of agricultural crops.

At the Krasnodar experimental station for plant protection, we used phytoncides against the vascular bacteriosis of cabbage. This disease is widespread and causes great damage to cabbage seed industry. This bacteriosis affects seriously the first year cabbage as well. In 1948, on some farms of the Pankovski, rayon, Krasnodar krai up to 30% of cabbage plants were destroyed by vascular bacteriosis.

The disease is usually transmitted through seeds and soil. Applying adequate grass crop rotation, only one source for generation of infection remains--theseeds. Generally, formalin and mercury preparations are used for disinfection of cabbage seeds.

The first experiments on disinfection of cabbage seeds with phytoncides against vascular bacteriosis took place in 1946 with the "Slava" variety.

The method of seed treatment with phytoncides is very simple: the onion ("Strigunovskii" variety) was grated and the paste was immediately mixed with cabbage seeds by way of shaking them for 10 minutes in a tightly closed jar. The onion had to be grated rapidly in order to prevent waste of volatile phytoncides particularly active during the first minute after thier isolation. 26 g. of grated paste were taken per 100 g. of seeds. The seeds remained an hour in the closed jar, after which they were spread in a thin layer for dryinging; in 6 hours they were already dry.

For comparison, seeds treated with formalin were taken. Part of the seeds were left without treatment (control). All the seeds were sown in beds without treatment (control). All the seeds were sown in beds on April 24; germination was normal. Cabbage seedlings were planted on June 3, in a plot occupied in previous years by sunflowers and corn.

The development of vascular bacteriosis in 1946 was slight. The following results were obtained at the record taking during the harvest on October 18 1.9% of plants were affected with vascular bacteriosis after the seeds were treated with formalin and 1.8% after seed treatments with phytoncides; among control plants, 8.2% were diseased.

Thus, treatments of cabbage seeds with onion phytoncides gave results similar to those of formalin treated seed.

In 1949 the experiment was repeated on an early cabbage variety "Number One", which is more susceptible to vascular bacteriosis. In order to obtain phytoncides, onion (shalot variety), garlic and horseradish root were selected.

The method of seed treatment was similar to that of 1946.

The sowing in hotbeds (cold frames) took place on February 3; each lot had a hotbed. Germination of cabbage seeds was similar in all the lots (from 83-87 %).

Normal simultaneous germination was noted in hotbeds. On April 7-9, the cabbage was planted in the ground of an irrigated polot. In 1948 this plot was occupied with tomatoes; in 1947, with alfalfa. Each lot had an 800 square meter area divided by irrigation canals into ten plots.

Appearance of vascular bacteriosis was recorded on June 10 in the first few plots of all the lots.

The first records of affection of cabbage with vascular bacteriosis were taken on June 14 (table 1)

During harvesting, the final record-taking was carried out and it appeared that the disease was observed in all the lots only in the first five plots and, in the other five plots, all the plants were healthy. This was probably due to the uneven irrigation (the last plots were less well irrigated than the first ones).

After the harvesting, 1348-1507 plants were analysed in each lot (table 2).

Table 1 (p.52)

Lot and Treatment Agent	Number of Cabbage Plants: Total	Number of Cabbage Plants: Of which were affected	% of Affection
Control	277	64	23.1
Onion	290	28	9.7
Horse radish	269	12	4.4
Garlic	270	4	1.5
Formalin	288	6	2.1

Table 2 (p. 52)

Lot and Treatment Agent	Number of analyzed plants	% of affection in various lots: 1	2	3	4	5	Mean Percentage of affection
Control	1387	28.6	19.7	[illegible]	13.7	4.1	15.4
Onion	1473	6.8	19.1	[illegible]	4.7	1.2	8.1
Horse radish	1348	17.4	6.1	2.5	6.3	1.3	6.7
Garlic	1398	6.9	5.6	1.0	2.6	0.6	3.3
Formalin	1507	10.4	1.6	2.4	1.4	1.5	3.5

It is seen from the given reports that the treatment of cabbage seeds with phytoncides conditioned the lowering of the degree of their affection by vascular bacteriosis. Of the three tested plants, the onion had the least toxic phytoncides. Best results were obtained from garlic treatment of the seeds. The toxicity of garlic phytoncides was no less strong than that of formalin.

The method of treatment of cabbage seeds with garlic phytoncides must find a wide field in Sovkhozs and Kolkhozs because it is simple, sufficiently effective and does not affect the germination of seeds. Besides, it is harmless for humans. At a standard cabbage seed consumption of 400 g. per hectare, there will be need of only 100 g. garlic for the disinfection of seeds.

Dubrova, G. B. [Effects sof garlic phytoncides on mold fungi] Mikrobiologiia 19:229-234. May/June 1950.

Up to the present time, little has been published on the study of the effects of phytoncides on mold fungi. At the same time this problem is of undoubted interest for the clarification of phytoncides' role in the life of the plants themselves (in connection with the hypothesis of the protective significance of phytoncides), as well as for a possible use of phytoncides in suitable practical fields.

The purpose of our research was to follow up the effects of garlic phytoncides on some representatives of mold fungi. We selected as objects such species of mold, which are widely spread in the nature. We studied Aspergillus niger, Asp. oryzae, Penicillium glaucum. Pen. notatum, Mucor racemosus, Rhizopus nigricans, Oidium lactis, Fusarium, Garlic (Allium sativum) was taken as a source of phytoncides.

On the surface of wort-agar, poured in Petri dishes, spores of various mold fungi were sown. For the sowing, 0.1ml of wort with an equal amount of spores for all the species, was taken. With a sterilized cork borer, 9mm in diameter, a well was made in the agar, in which a garlic paste was placed in the amount of 250 mg, and prepared on a grater from a garlic bulb. The dishes were placed in the incubator at a 28° temperature. On the first-second day a growth of mold fungi was observed. Around the well there was no growth - a sterile zone was formed, different for various molds; the amount of garlic was similar. The mean diameter of sterile zones for various fungi is given in Table 1; the diameter was measured when the mold fungi began to form reporductive bodies. For Rhizopus nigricans the sterile zone diamter was measured during the first 24 hours of growth, because on the second day, the sterile field was covered with the mycelium of the fungus.

We estimated the fungicidal strength of garlic according to the size of the sterile zone. Table 1 indicates that the garlic affects most strongly the Fusarium and the Oidium lactis, slightest - the Mucor racemosus and Rhizopus nigricans.

Along the edge of the sterile field a non-spore forming zone was observed. Its width varied for different fungi.

The methods of the preceding experiment can be slightly changed by depositing a drop of garlic juice on the agar-wort surface and, by inclining the dish, forcing the drop to run off the agar surface. Then, along the edge of the drop trace - as in the first case - the sterile and non-spore forming znes and the reproductive zone be seen.

Microscopic research of the non-spore forming zone reveals considerable morphological changes. For example, there are thickenings at the ends of my celial hyphae in Asp. oryzae, but no reproductive bodies are formed. Conidia are completely lacking in the zone, while in the preparation taken from the edge, the usual amount of spores can be observed. In the non-spore forming zone of Asp. niger (fig. 4), as well as of Asp. oryzae the conidia have frequently a thickening at the end with a deformed segmentation. Its mycelium is well defined and septate and has a clearly visible granulated protoplasm. Normal fertility bodies and conidial are also lacking in the zone. Pen. notatum glaucum, Rhizopus nigricans do not form spores in this zone either and at the ends of the myceliumthere are thickened segmentations.

On the third day in Mucor racemusus zygospores appear, in the zone where at the beginning of the experiment there was no growth. In the preparation from the edge of the dish zygospores are lacking Oidium lactis forms in this zone a widened mycelium, with a granulated protoplasm; it does not break down into oidia (fig 8)

Thus, under the influence of garlic phytoncides, first of all, the formation of normal reproductive bodies is inhibited. Besides that, the mycelium is usually widened, septate and granularity of protoplasm is observed.

The possibility of a hereditary fixation of these changes, represents the topic of our subsequent studies.

We observed in this series of experiments, that 3-4 days later a complete growing over of the sterile zone takes place. This occur either due to further development of the mold fungus mycelium, which was already there, or due to late germinating of spores, the develpment of which was at first inhibited by the effects of garlic phytoncides, i.e. a problem arose, whether the latter have a fungicidal or fungistatic effect.

In order to solve it we performed another series of experiments. Their procedure consisted in the following. A glass ring with a 14 mm inner diameter and 6mm high was set on the slide. On top of this ring was placed the cover glass, with the previously applied drop of wort with a spore suspension. The number of spores in such a drop, in all the objects, was 200-250. Then inside the ring, 100 mg garlic paste was placed on the slide. To prevent drying out of the drop, the ring was smeared on both sides with vaseline. The preparation for control was similar, but without garlic. The test and the control preparations were placed in an incubator at 28°. In this arrangement of tests, the spores of Aspergillus niger, Asp. oryzae, Pen. glaucum, Pen. notatum, Mucor racemosus, Rhizopus nigricans, Oidium lactis, Fusarium did not germinate. At the same time the usual germination of spores took place in the control: swelling, formation of mycelium; spore bearing.

This was the reason for the assumption that the volatile fractions of garlic phytoncides, in amounts which were used in our tests, possess fungicidal properties.

We decided to find out, further, at what minimum exposure to volatile garlic fractions similar effect is produced. Procedures in this series of experiments were the same as in the preceding case, but the cover glass with the hanging drop was kept only for a short time over the 100 mg of garlic and then placed over another ring, without garlic. It was found that a 30-45 second exposure to volatile fractions of garlic phytoncides is sufficient for non-germination of Fusarium, Pen. notatum spores.

Table 3 gives an idea of time necessary to effect, by volatile garlic fractions, the spores of various fungi to the point when they do not germinate.

All this was carried out with a garlic paste prepared before the test.

Besides that we tested the fungicidal effect of a garlic past which was exposed to the air for 24 and 48 hours. In Asp. niger the fresh garlic paste killed spores, during the first hour after its preparation, on the average in one minute; paste exposed to air for 24 hours - in 4-6 minutes; paste exposed to air for 48 hours achieved the same effect in 8-12 minutes. Thus it was found, that the phytoncides of the freshly prepared garlic paste have the strongest effect.

It was noticed in observing the preparations with microscope that the gradual time increase in exposure of spores to garlic phytoncides, leads to considerable morphological changes. Thus, for example, the Asp. niger spores, after a 15 second exposure to freshly prepared garlic paste, did not develop normal fertility and 15-25% of spores did not germinate. The mycelium of the germinated spores was changed considerably; granularity of protoplasm, swelling and septation of hyphae were observed. After a 30 second exposure 40-60% spores germinated. The mycelium was more swollen and septate. No reproductive bodies were formed. One minute exposure stopped spore germination. Thus, small doses of volatile fractions of garlic phytoncides acted fungistatically, large ones - fungicidally.

In the following series of experiments, the effect of volatile garlic fractions on mycelium and dry spores was studied. As a result of the tests it was found that, in comparison with the germination of spores, a 1-1/2-2 times longer exposure of mycelium is needed in order to stop its growth and a 30-240 times longer one of dry spores. Thus for Pen. notatum a four-hour exposure to 100mg of garlic is needed to kill dry spores. For asp. niger the time required is one hour. Thus the most resistant to garlic phytoncides are the dry spores, the most sensitive - the germinating spores.

The next stage in our work was the study of tissue juices of garlic.

For this experiment we took sterile garlic juice and diluted it with distilled water. Then to each ml. of each solution we added 1mg of wort with a suspension of spores from 800-1000. The control was prepared without garlic. The apparatus itself was placed in the incubator at a 28° temperature. Observations were made every other day during 10 days. On the third day sowing in dishes with wort-agar was carried out. Table 4 gives an idea of garlic juice dilutions at which the sowings in the dishes do not grow.

It should be noted that lesser concentrations of garlic juice have a lesser effect on mold fungi. In sowing in dishes it is observed that the growth of Oidium lactis with a 1 : 1600 dilution will give 640 colonies, of Pen. notatum, with a 1:1600 dilution - 350 colonies.

Corresponding control dishes produced a continuous growth of culture. The process of mold development is inhibited when the concentration of garlic juice is increased. Thus, for example, in a 1600 times dilution, the development process in *Aspergillus niger*, in relation to control, is delayed for 24 hours; when the juice is diluted 800 times - for 5 days. A 400 times dilution delays the mycelium formation for 6 days and in this case there is no formation of conidia. Analoguous results are obtained with other mold fungus species as well. Thus the tissue juice ofgarlic, with the density of spores as we had in our experiment, acts in small concentrations predominantly fungistatically and in large - fungicidally.

Besides garlic, strong antifungal properties are also present in phytoncides of onion, mustard, cherry laurel, laird cherry (*Cerasus padus*). However, the elaboration of this material is not part of our article's purpose. This study was carried out at the base of the phytoncide laboratory of the Institute for Experimental Medicine AMN, SSSR (Akademy of Medical Sciences, USSR)

I feel it my duty to express my deep gratitude to Professor B. P. Tokin and N. A. Shtern.

Conclusions

1. Garlic phytoncides in high concentrations act fungicidally, in low fungistatically.

2. Various species of mold fungi are of various sensitiveness to garlic phytoncides. The most sensitive ones are *Fusarium*, *Oidium lactis*, *Pen. glaucum*; the least sensitive - *Rhizopus nigricans*, *Mucor racemosus*.

3. Garlic phytoncides affect strongest the germinating spores of mold fungi and slightest - the dry spores.

4. The fungicidal and fungistatic effects of garlic phytoncides become lower corresponding with the duration of garlic paste's exposure to the air.

Miazdrikova, M. N., [The effect of "phytoncides on storing carrots]. Sad i Ogorod (Orchard and garden). 1950. No. 9, pp. 51-52.

The phytoncidal effect of onion and of pine and fir needles on many micro-organism has been establishe d(Prof. B. P. Tokin). For the control of carrot diseases we studied the effects of onion juice and aqueous extracts of onion, pine, and fir on the Sclerotinia, Botrytis and Alternaria fungi.

The observations were carried out first under laboratory conditions. on pure cultures of these fungi and then in storage places.

Most effective against carrot diseases were the juice and the aqueous extract of onion. Aqueous extracts of pine and fir needles produced less satisfactory results.

The aqueous extracts were prepared from cut juicy onion skins of sharp and semi sharp varieties, which were taken in the amount of 7% of the water weight; extracts from pine and fir needles were taken in the 4-10% ratio. Soaking continued from 1-5 days at a + 14 to 4° temperature. The lower the temperature, the longer the soaking period.

Observations were carried out on different carrot varieties (shantene, Nantskaia, Valeriia) which came in for storage from the moscow oblast.

Two methods were applied in order the check the effects of phytoncides on preservation propeties of carrots; a) immersion into an aqueous extract and b) spraying of carrots. In the first case the carrots were immersed for 10 minutes into aqueous extract of onion or pine needles, and, without drying them previously, they were stored; as a result, the affection of carrots with Botrytis, Sclerotinia and Alternaria fungi was 2-3 times lower than that of carrots which were not exposed to phytoncidal action (Table 1)

Due to the cumbersomeness of the immersion method, it was decided to replace it with sparying.

Spraying of carrots with onion extract before storing them produced an almost 3 times lower disease affection. Each row of carrots being stored in boxes or stacks (stock piles?) was sparyed.

According to our observations, it is quite possible to use the non-standard and even partly diseased onions for the preparation of aqueous extract of onions; in using diseased onions the healthy part should be taken, mainly the germ part which - as it is known - possesses a stronger phytoncidal property. For spraying of one tone of carrots, about 10 liters of water and 700 g. of onions will be needed.

Thus, observations demonstrated, that phytoncides of onion, pine and fir needles, are entirely effective for the control of carrot diseases during storage.

Razumovich, M. B. and Naumov, S. M. [Effect of Onion, garlic and bird cherry (Prunus avium) on seeds of higher plants]. Priroda 40(4):64-65. Apr. 19[illegible].

We used for the experiments the seeds of winter rye (Novozybkovskaia M-4) and spring wheat (Lintescens 0-62). The experiments started in December 19[illegible] and terminated in May 1950. The research procedure was as follows. The plant material which served as a source of phytoncides, was weighed in 2 and 5 g. amounts and cut into small pieces; 30 cubic cm. of water were added to each eighed portion. Then the stirred paste was introduced quickly into petri dishes, where there were already the previously selected seeds of the indicated plants, in amounts of 100 grains in each dish. The Petri dishes were covered with sheets of heavy paper in order to reduce the evaporation of moisture. Control experiments were carried out simultaneously. Checking of seed germination took place after 4-5 days.

Experiments indicated that of the plant material which we used the most inhibiting effect on seed germination was produced by bird cherry; it is followed by garlic and then onion. (see table).

We also discovered the inhibiting effect of volatile fractions of phytoncides of garlic and bird cherry on seeds of the plants mentioned. The treatment of seeds with volatile fractions of phytoncides was carried out in the following manner: garlic and bird cherry were carefully grated and this grated paste, in the amount of 15g., was placed in a tightly covered container in which were already Petri dishes with rye and wheat grains (one hundred grains in each dish).

Table (p. 65)

Amount of germinated seeds (per 100) exposed to effects of bird cherry, garlic and onion phytonicides.

	Experiment with bird cherry			Experiment with garlic			Experiment with onion		
	Weighted portion of plant material		control	Weighed portion of plant material		control	Weighed portion of plant material		control
	2g.	5.g		2g.	5g.		2g.	5g.	
Winter rye	6	o	92	15	2	94	26	21	9[illegible]
Spring Wheat	53	6	91	67	8	95	73	36	9[illegible]

It was disclosed, as a result of experiments, that the inhibiting effect on seed germination was clearly manifest already after a 10-15 minute exposure, but this effect was considerally more pronounced after a 2 hour and particularly - a 4 hour treatment of the seeds.

It is interesting to note, that not only the stem length, but also the length of the roots of the germinated rye and wheat seeds, exposed to the effects of tissue juices and volatile fractions of phytoncides of garlic and bird cherry, was almost half the length of the control plants.

We suggest, that the experimental data, obtained in studying the effects of phytoncides on seeds of higher plants, can be used as an indicator for the development of the phytoncidal strength of various plants; the amount of germinated seeds and the energy of their growth have to be taken into account.

Zaitsev, G. N. [Effects of phytoncides of bird Cherry buds on fungi]. Priroda 40(9):58. Sept. 1951

B. P. Tokin's research disclosed that the bird cherry Padus racemosa (Lam.) S. K. is very toxic for various species of protozoa and insects.

In order to find out about the effects of bird cherry phytoncides on fungi the imperfect fungus Penicillin sp. and a domestic fungus Coniophore cerebella were taken. The experiments took place in Leningrad, in January-February 1951.

The procedure was as follows:

Test-tubes with an agar slant were inoculated with fungi. Then in each test-tube, with the exception of control tubes, were introduced 1.5 g. of bird cherry buds, ground in a mortar. The experiment lasted 36 days. During 12 of them, the test-tubes were in an incubator, at a /25° C. temperature.

In the control tubes the fungi cultures developed normally and in the experimental ones there was no growth of the cultures. An interesting fact is that the buds possessed phytoncidal properties when they were opening.

In order to stimulate the opening of the buds, the branch was placed for 30 minutes in water; the temperature of the surrounding air was +45+C. When it was done, the buds of the bird cherries did not show any fungicidal action

Gorlenko, M. V. and Shneider, Iu. I., [On the biological role of phytonicdes of higher plants]. Zhur. Obshch. Biol. 12:363-367. Ref. Sept./Oct. 1951.

[This paper deals mainly with a review of the role of phytonicides in the immunity of plants to microorganisms. The concluding remarks are quoted.]

Thus our experiments, as well as data of the literature, speak of the fact that phytoncides of a given plant have a destructive effect only on microorganisms not adapted to parasitizing that particular plant.

What then is the tole of phytoncides in the formation of protective properties of plants? We think that it consists in the following:

(1) The pathogenic microflora of one or another plant is not permanent. All the time saprophytic forms, which come in touch with plants, are adjusting themselves to parasitizing these plants. Phytoncides are one of the factors resisting or restraining this process. Probably only a few bacterial cells or fungus spores overcome the plant resistance by originating new parasitic microorganisms

2) Microorganisms affecting each plant are not equal in degree of parasitism--among them is a large group of semi-parasites which affect only weakened plants such as those which are completely or partially lacking protective properties. In this case the presence or absence of phytoncides can determine the parasitizing potentiality of micro-organisms of such type.

In creating plant varieties resistant to organisms which are already adjusted to parasitizing, new (resistant) varieties must have a completely altered protective apparatus. Therefore, the qualitative status of phytoncides is not a permant one; it is probably different among varieties of varied resistance.

It is not correct to assume that only the presence or absence of phytoncides determines the resistance of plants to diseases. Phytoncides cannot be examined alone. Besides the fact that producing of phytoncides depends on the condition of the plant and the latter is a function of environment, in the pehnomenon of resistance (immunity) the entire plant organism participates.

Naturally the attempts to explain, let us say, various degrees of susceptibility of Aurantiaceae to bacterial necrosis by the presence or absence of phytoncides are inadequate (Khetagurova, 1950).

Thus, phytoncides, as a biological phenomenon, have to be considered only in connection with the general state of plants which produce them.

Kharkina, G. A., [Comparative effects of phytoncides from garlic on phytopathogenic bacteria]. Mikrobiologiia 20:434-437. Sept./Oct. 1951

The priority of discovery of antibiotic properties of highest as well as lowest plants belongs, as it is known, to Russian scientists. Antibiotics of highest plants were discovered in 1928 by Tokin and were named by him - phytoncides. According to this definition they are a group of substances of plant origin, diverse in their chemical make-up, but united by the commonness of their effect on other organisms; all the phytoncides have a more or less vigorous protistacidal and fungicidal action. This article is dedicated to the study of effect os phytoncides on phytopathogenic bacteria. In this study, natural garlic and "sativin" preparation obtained from garlic by the method accepted in Tokin's laboratory - was used as a source of phytoncides. The effects of volatile and non-volatile fractions of phytoncides of garlic and "sativin" on representatives of the following groups of phytopathogenic bacteria were studied;

1) Causal agents of plant rots (Bact. carotovorum, Bact. phythophthorum); 2) Bacteria which cause spots on leaves and form yellow colonies in nutrient media (Bact. malvacearum, Bact. vesicatorium, Bact. heteroceum); 3) fluorescent bacteria, pathogenic for plants (Bact. atrofaciens, Bact. citriputeale, Bact. xanthochlorum); 4) Bact. tumefaciens, causing root canker (crown gall) of plants.

Among the research subjects was also included the bacteria isolated as a pure culture from affected garlic heads ("garlic" bacterium). According to its cultural and biochemical properties it is found to be close to representatives of the group of causal agents of plant rots of the Bact. carotovorum - Bact. phythophthorum type.

Methods

Procedures were applied which were adapted for analogous research in Tokin's laboratory (2). The effects of volatile fractions of garlic paste and "sativin" on bacteria sown in Petri dishes were tested. In all the tests a uniform ratio of garlic paste and "sativin" was taken. The results of the effects of the volatile phytoncide fractions were recorded after 24 hours according to the size of the sterile zones. Bacteriostatic or bacteriocidal effects of phytoncides were established by way of inoculation from the sterile zones onto the meat peptone agar media. The activity of the non-volatile phytoncidal fractions were studied by depositing a drop of "sativin" or garlic juice on the surface of the bacteria-sown agar in Petri dishes. The drop, in running off, formed a sterile "path." According to its width was calculated the relative degree of effect of the garlic and "sativin" phytoncides on phytopathogenic bacteria being studied. After the reaction to volatile as well as the non-volatile fractions of garlic and "sativin," the Petri dishes, sown with bacteria, remained 24 hours in an incubator at 2?°.

Comparative effects of volatile fractions of garlic and "sativin" on a number of phytopathogenic bacteria

Results of experiments pertaining to this section are in table 1.

Table 1 (p.435)

Comparative effects of volatile fractions of phytoncides of garlic and "sativin" on growth of cultures of various phytopathogenic bacteria.

Names of bacteria	Garlic			"Sativin"		
	Time of exposure in hours					
	1	24	48	1	24	48
	Area of sterile zones in cm.					
Bact. phythopthorum......	9.8	54.9	70.8	0	18.1	21.6
" carotovorum........	10.5	19.6	22.0	0	6.6	7.3
Bacterium isolated from garlic	0	15.9	17.8	0	4.9	5.96
Bact. malvacearum	16.2	46.0	53.4	0	19.6	21.2
" heteroceum.........	7.5	40.0	40.6	0	9.6	16.9
" vesicatorium......	17.6	44.9	64.0	0	12.0	14.5
" atrofaciens.......	2.13	70.8	70.8	0	18.9	26.0
" Citriputeale......	25.0	70.8	70.8	0	31.7	32.7
" xantochlorum......	28.3	44.5	46.5	0	7.5	10.5
" tumefaciens.......	0	42.3	45.4	0	6.14	7.06

Remark: 0- uninterrupted growth (lack of sterile zones); 70.8 - lack of growth (on the entire surface of the dish).

Following conclusions can be made on the bais of the data given in the table.

1. Volatile "sativin" fractions produce a depressing effect on all the tested bacteria species, but a considerably weaker one than the volatile garlic fractions. The apparent reasons are as follows:

1) As it is known (Tokin(2)), in obtaining the preparations chemically, only part of the active bacteriocidal properties of the phytoncides are extracted. And besides, they are apparently already in an altered state, as compared with the active substances which are created by plants in the process of their life activity.

2) As a result of imperfection and faults of the method being used for the obtaining of "sativin," the phytoncide extraction from garlic could have been incomplete.

2. Among the 11 tested species of phytopathogenic bacteria, the most resistant to the effects of the volatile fractions of garlic and "sativin" were the representatives of the group of plant rots' agents and the least resistant--the group pf plant parasites, which produce a yellow pigmentation. The group of fluorescent bacteria occupied an intermediate position. Within these groups single bacteria species manifested their individual resistance in relation to the effect on them of volatile phytoncide fractions.

3. Strength of action of the phytoncide of garlic and "sativin" depends on the duration of exposure and the amount of phytoncide material.

Thus, with the increase of phytoncide material (garlic as well as "sativin") the sterile zone area increases. With increased phytoncide material and longer exposure (2g of garlic and 24 hour exposure), after 9 days is observed not the usual decrease but an increase of the sterile zone. Decrease of zones with the time is explained by the resumption of growth of the inhibited, but not killed bacterial cells.

4. The effect of the volatile fractions of garlic and "sativin" on phytopathogenic bacteria, under the conditions of the adapted methods, was bacteriostatic and only in single cases---bacteriocidal. This is confirmed by the data in table 2

Table 2 (p. 436

Character of the effect produced by phytoncides on phytopathogenic bacteria

Names of bacteria	Time of exposure of volatile fractions in hours						Control
	Garlic			"Sativin"			
	1	24	48	1	24	48	
Bact. carotovorum	+	+	+	O	+	+	++
" phythopthorum	++	+	-	O	+	+	++
Bact. isolated from garlic	O	++	++	O	++	++	++
Bact. malvacearum	O	+	Very weak	O	+	[illegible]	++
" citriputeale	++	-	-	O	+	-	++
atrofaciens	++	-	-	O	+	-	++

Remarks:) - means uninterrupted growth, wihhout zones; ++ growth analyogous to the growth in the control; + slightly weaker growth than in the control; - lack of growth.

It is seen from table 2 that the bactericidal or bacteriostatic effects of garlic phytoncides depend onthe species of phytopathogenic bacteria and on the duration of exposure. The effect of the volatile fractions of "sativin" was bacteriostatic in the overwhelming majority of cases.

All the above-mentioned can be also illustrated with the biological spectrum of action. This experiment was carried out by following methods: In the center of the agar in the large Petri dish (18 cm in diamter) was cut a 1 cm wide channel. Perpendicular to it were streaked phytopathogenic bacteria of various species. In the channel was placed garlic paste or "sativin." According to the distance from the edge of the groove to the beginning of the growth of the culture, the relative degree of sensitiveness of the given bacteria species to phytoncides could be estimated.

Comparative effects of non-volatile fractions (juices) of garlic and "sativin" on phytopathogenic bacteria.

Results of experiments pertaining to this problem are in table 3.

The obtained data lead to the following conclusions:

1. The effects of phytoncides of the garlic juice are stronger than the effects of "sativin."

2. The strength of phytoncide effects on different species of phytopathogenic bacteria varies. The group of bacteria with yellow pigmentation appeared to be the least resistant to the effects of phytoncides.

3. The effects of phytoncides of garlic juice and "sativin," under the conditions of the methods applied, is characterized as bacteriostatic.

The strength of the phytoncide effect depends on the degree of their dilution, which can be seen in table 4.

The following has also been clarified in the process of the work:

1) The volatile phytoncide fractions are absorbed by the nutrient media; 2) a diffusion of phytoncides takes place in the nutrient media; 3) volatile fractions of garlic and "sativin," stop the movement of bacteria or change its character; 4) in the process of preserving of garlic and "sativin" the activity of their volatile and non-volatile fractions decreases.

In the left dish were sown (downwards); Bact. phythopthorum, Bact. tumefaciens, Bact. xantochlorum, Bact. citriputeale. In the right dish (downwards); Bact. vesicatorium, Bact. campestre, Bact. malvacearum, Bact. heteroceum.

Table 3 (p. 437)

Effects of non-volatile phytoncide fractions on Phytopathogenic bacteria

Names of bacteria	Garlic	"Sativin"
	Width of the sterile "path" in cm.	
Bact. phythophthorum.	1.1	0.6
" carotororum....	0.7	0.2
Bacteria isolated from garlic.............	0	0
Bact. vesicatorium...	3.2	1.6
" malvacearum....	3.4	2.0
" heteroceum.....	1.5	0.9
" xanthochlorum..	1.0	0.8
" atrofaciens....	1.8	1.2
" citriputeale...	2.8	1.4
" tumefaciens....	0.8	0.1

Table 4 (p. 437)

Influence of the degree of phytoncide dilution on its effects on bacteria

Names of bacteria	Garlic juice				"Sativin"			
	Un-diluted	1:10	1:100	1:1000	Undiluted	1:10	1:100	1:1000
	Area of sterile zones							
Bact. phythophthorum.....	5.75	3.4	0	0	5.5	0	0	0
" tumefaciens.........	11.4	6.5	4.35	0	0	0	0	0
" citriputeale........	9.4	5.95	0	0	4.5	0	0	0

In closing I want to express my deep gratitude to Prof. F. V. Khetagurov for guidance and assistance in carrying out this work at the Institut for Applied Zoology and Phytopathology.

Conclusions

1. All the phytopathogenic bacteria studied are to some degree subject to the effects of garlic and "sativin" phytoncides. And the effects of garlic phytoncides are stronger than those of "sativin."

2. A greater resistance can be noted among the phytopathogenic bacteria pathogenic to humans and animals. It can be explained by the greater adaptability of phytopathogenic bacteria to life in plants and to factors of their specific exterior surroundings.

3. The disclosed effects of phytoncides of garlic and "sativin," in tests on a number of phytopathogenic bacteria, indicate the necessity of further research on utilization of phytoncides in the control of bacterial plant diseases.

Lesnikov, E. P., [Antifungal effects of phytoncides.] Priroda 40(10):56-58. Ref. Oct. 1951

We exposed 17 strains of pathogenic fungus cultures to various effects of phytoncides; Epidermophyton K.-W., snow-white, gypseous and violet Trichophytons, rusty and fluffy (downy) Microsporum, Achorion Sch., Geotrichoid, Candia albicans, C. triadis, pinky yeast, Forula and Hormodendron. It has been disclosed, that in comparison with garlic, the volatile fractions of onion have a stronger fungistatis effect in shorter time, while the fungicidal effect of garlic is more clearly manifest than in onion. (Table 1)

Table 1 (p. 56)

Effect of volatile fractions of onion and garlic paste on pathogenic fungi

	Volatile fractions of onion paste		Volatile fractions of garlic paste	
	Fungistatic action	Fungicidal action	Fungistatic action	Fungicidal action
	Time of exposure (in hours and minutes)			
Epidermophyton K.-W.	1 hours	50 hours	2 minutes	20 hours
Trichophyton gypseum	20 hours	50 hours	3-20 hours	20 hours
Trichophyton niveum	3 hours	20 hours	10-20 hours	20 hours
Trichophyton violaceum	10 minutes	15 minutes	1 hour	5 hours
Achorion Schönleini	5 minutes	20 hours	15minutes	20 hours
Microsporum ferrugineum	5 minutes	25 hours	10 hours	24 hours
Geotrichoides	1 hours	Not obtained within 72 hours	48 hours	72 hours
Candia albicans	5 minutes	3 hours	2 hours	24 hours
Rhodotorula	30 hours	50 hours	20 hours	48 hours

Table 2 (p. 57)

Effect of onion and garlic juices on pathogenic fungi

Cultures	Fresh onion juice		Fresh garlic juice	
	Fungistatic action	Fungicidal action	Fungistatic action	Fungicidal action
	Time of exposure (in hours and minutes)			
Epidermophyton K.-W.	1 hours	Above 1 hour	15 minutes	20 minutes
Trichophyton niveum	30 minutes	24 hours	6 minutes	20 hours
Trichophyton violaceum	2 minutes	25 minutes	--	2 minutes
Achorion Schönleini	--	2 minutes	--	2 minutes
Microsporum ferrugineum	2 minutes	2 hours	10 minutes	1 hour
Geotrichoides	20 minutes	20 hours	30 minutes	1 hour
Rhodotorula	2 munites	48 hours	6 minutes	5 hours

The garlic juice appears to be more active in various time periods, than the onion juice, both in fungistatic and, particularly, in fungicidal effects. The squeezed out paste and the alcoholic extract of onion indicated also a clearly manifest antifungal action (table 2). Preliminary spraying of the nutrient media with garlic juice and also with "sativin", either completely stopped, or partly inhibited the growth of fungi.

In a book published in 1947, V. Kuprevich (5) reports on the effect of fresh extracts from oats and potato leaves on germination of uredospores of Puccinia coronifera Kleb. If in an extracts of four oats varieties (plant which P. coronifera frequently parasitizes) the spore germination percentage fluctuates between 15-45, then in leaf extract of four potato varieties (plant which ordinarily is not a host for parasites) no growth at all was observed.

R. Vasudeva (27) and B. Choma (21) found that in presence of juice of a foreign host-plant, the spores of fungi-parasites of onions Botrytis Allii and parasites of apple-trees Monilia fructigena, Fusarium coeruleum (lib.), Phytophthora erythroseptica and Pythium sp. - cannot germinate. This could explain the resistance of onions to apple-tree parasites and the resistance of apple-trees to onion parasites. In 1948, I. Zagaievskii (5) comparing a number of fungicidal measures found that the most effective one against the causal agent of epizootic lymphangitis Cryptococcus farciminosus was fresh garlic juice. In 1949, Tsimerinov, checking the effect of volatile substances of garlic on culture of fluffy Microsporum and on fungal elements of the hair, could become convinced in the following: at a 3 hour exposure the growth of the culture was delayed for 2-3 days as compared with the control one; at a 6 hour exposure there was no growth; hair fragments were growing at a 30 hour exposure, but at a 34 hours exposure there was no growth. According to his data, the sowing of hair fragements affected with fluffy Microsporum, after 5 hours in fresh garlic juice resulted in an inhibition, and after 9-1/2 hours - in a lack of growth of the culture. In 1949, Sheniakin and Khokhlov described their observations of allicin - acyclic derivative of garlic. In experiments with Aspergillus niger, Penicillium notatum, Trichophyton gypseum and Microsporum Audouini, the antifungal properties of allicin are confirmed, and the fungicidal concentrations of allicin are about 10 times higher than the fungistatic ones.

Dubrova (4) was investigating the effects of garlic phytoncides on cultures on 8 mold fungi and came to the following conclusions. The effect of volatile fractions of garlic juice was being tested. Large concentrations of garlic phytoncides act fungicidally, small - fungistatically. Varéous sensitivity was disclosed in various fungi (the most sensitive ones are: Fusarium, Oidium lactis Penicillium glaucum; the least sensitive ones are: Rhizopus nigricans, Mucor racemosus). The effects of phytoncides inhibit the formation of reproductive bodies. Fungi were not growing when garlic juice in a 1:200 (mucor racemosus) and 1:6000 (Fusarium sp.) dilution was introduced in dishes.

Tokin, B. P. [Destroyers of microbes - phytoncides]. Gosizdat kul'turno-pros... literatury. Moscow, 1951. (128 pages)

Phytoncides and Food Industry (p. 121)

Could phytoncides be used for storing of meat, fish, fruits, vegetables?

Yu. A. Ravich-Shcherbo suspended on wires in glass containers under the cork specimens of fresh fish - a kind of sprat. On the bottom of these containers were placed various phytoncide sources; chopped horse-radish roots, grated onion or garlic bulbs, mustard prepared 24 hours before or longer. Nothing besides warm water was added to mustard. The well-studied chemical process begins. Vapors of the so-called allyl-mustard oils formed. The glass containers - as it is clear from the above - were closed with corks. The experiments were carried out at a 15-17° temperature. Control specimens of fish were placed in exactly similar containers, but contrary to the experimental specimens, they were not exposed to volatile phytoncide sources. The latter were supposed to produce some antiseptic effect on putrid bacteria and mold fungi which might always be found on the surface of fish skin or inside the fish tissues.

For 13 days Ravich-Shcherbo observed the results of the experiments.

Is it necessary to describe in details what was happening to the control fish? Already after 4 days the fish was covered with a heavy layer of bacteria in disintegrating tissues seen with the naked eye. After 6 days the fish was so decomposed, that it fell off the wire. It was impossible to separate the skin from the meat, all the tissues became pasty, the smell was strong and putrid.

The fish under horse-radish and garlic vapors was not fresh either, but the rotting process (especially due to garlic) was inhibited considerably. The smell was putrid, but the fish surface was almost slimy, the meat was quite solid, the sking could be taken off with difficulty.

Absolutely amazing was the effect of mustard vapors: the fish looked well, there was no slime, the color was that of a fresh sprat. There was no putrid smell; the meat was solid, not pasty.

Tests were made on bacteria also: nutrient media were inoculated with bacteria from the surface of the sking and from the "depth" from sprat tissue.

On the 13th day of experiments with the sprat, which was exposed to mustard vapors, almost no bacterial germs were discovered, as if the sprat were canned.

Of no lesser interest are G. B. Dubrov's experiments with beef meat. Under non-sterile conditions (without prevention against bacteria and molds) a few grams of the meat were hung on a hook fastened to the cork of a 1/2 liter glass container. On the bottom of the container were placed phytoncide sources - chopped parts of one or other plants.

As a control served meat which was placed in a similar container, butnot subject to effects sof volatile phytoncides. The temperature was similar in all cases of experiments. The containers were closed tightly, in order to prevent entering the air of new bacteria and spores of fungi.

On the 3 - 5th day the meat in the container without phytoncides begins to mold and rot profusely. An abundant malodorous slime appears on the surface. The meat exposed to volatile phytoncides of garlic horseradish and to mustard vapors, even after 5 days shows no traces of rotting and molding. The meat which was exposed to effects of horse radish and mustard phytoncides, did not differ in color from the control meat. The meat which was exposed to volatile phytoncides of onion, was slightly moulded, but the rotting process was inhibited.

Observations with the naked eye of some meat pieces continued during a year.

In other containers a thorough analysis, including the study of amounts and species of bacteria and molds, was performed on the fifth day, after 2 weeks, half a year and a year.

After a month there was of course no need to continue observations of the control meat. Strictly speaking there was no meat, there was a black stinking slime - remnants of a decayed, fallen from the wire, meat.

The piece of meat which was in the atmosphere of volatile horse-radish phytoncides began to mold and rot after six months. This means, that either not all the fungus spores were killed at the very beginning, or, that in spite of precautions the latter entered later, when the secretion of antifungal substances was long ago stopped.

The meat which was in vapors of garlic phytoncides and in mustard vapors was not subject to putrid decomposition, but its color changed. And what happened after a year? A rotting of meat, which stayed exposed to horse-radish phytoncides, was playing havoc. The mat, which stayed exposed to vapors of garlic phytoncides, was covered scarcely with mold mycellia.

Absolutely stunning results - hard to believe without a personal experiment - were obtained with pieces of meat placed in an atmosphere of volatile phytoncides of cherry laurel leaves and mustard vapors. Even after a year there were no traces of meat decay. A microscopic section was cut and there was proof, that even the finiest tissue structure was preserved.

What actually happens? It is clear that the powerful phytoncides, killed at the beginning of the experiments, all the bacteria and molds which were on the meat as well as on the walls of the containers. Later on, due to tight closing of containers the entering of bacteria and fungi from the air was prevented.

Unfortunately, these experiments are not yet a guide for action and have so far only a scientific interest.

On August 3, 1948, Sukhachev placed a freshly prepared paste of grated horse-radish on the bottom of a glass container, and on the dividing wall, in the approximate center of the container, he placed branches with gooseberries and with white, red and black currants. He greased the edges of the container with vaseline and closed it tightly with a cover. (Fig. 35 [p. 126].

At a 18-20° temperature the berries did not spoil for five months! However, if the container is opened even for only a few minutes, the berries begin to mold. This is understandable, because the volatile horse-radish phytoncides probably kill the bacteria and mold fungi during the first minutes and hours and after that their production is exhausted.

D'iachenko, P. F., and Romanovich, T. G., [Bactericidal properties of protamines] Mikrobiologiia, vol 21, No. 2, 1952. pp: 185-186

We received for our study, from the department of organic and biological chemistry at the Moskva Rybvtuz, protamine-sulfate from sturgeon milt (sturin).

We treated the aqueous solution of protamine at pH: 3.8 with caustic baryta [barium hydroxide] in order to free it of sulfuric acid. The residue of barium sulfate was eliminated by filtration. The free protamine solution obtained showed a negative reaction to sulfuric acid and barium and the reaction was strongly alkaline (above pH:10.0).

Further, the protamine solution was neutralized with hydrochloric acid up to pH:6.9, and was tested for bacterididal properties. For the study of sensitiveness of various microorganisms to the effects of protamine, we sowed in Petri tubes a [film] of the culture being tested. The agar was prepared in Bogdanov [2] media with casein hydrolystate. Following the congealing of agar, on it were set vertical sterile short (10-12 mm) hollow tubes, in which protamine solution was introduced with a pipette.

After 3 days of incubation, with adequate optimal temperature on a well developed surface [growth] of the culture being tested, the zones of growth inhibition were clearly outlined around each little tube. We discovered very clearly the sensitiveness to protamines of a number of gram-positive and gram-negative bacteria: Bac. subtile, Bac. mycoides, Bac. mesentericus, Bact. coli commune, Streptococcus lactis. The protamine inhibits Bac. subtiles, Bac. mesentericus Bac. mycoides and Bact. coli commune.

In affecting the lactic acid streptocco, a certain growth stimulation of the streptococci is noticeable around the metallic tubes. In relation to the mold fungi it appeared that protamine has no effect whatever on Oidium lactis and Penicillium glaucum.

CONCLUSIONS

Bactericidal properties of protamines (sturin) were studied. It was established, that sturin inhibits some microorganisms (Bac. subtilis, Bac. mycoides, Bac. mesentericus, Bact. coli commune) and stimulates the growth of other lactic acid bacteria (Str. lactis).

Sturin does not manifest any antibiotic properties towards the mold fungi Oidium lactis and Penicillium glaucum.

Goriachenkova, E. V.

Ferment chesnoka, obrazuiushchii allicin (alliinaza) - proteid fosfopiridoksalia.

Garlic enzyme producing allicin (alliinaze) - proteid of phosphopyridoxal.

Doklady Akademii Nauk SSSR. 87(3) :457-460. November 21, 1952. 511 P444A

(In Russian).

GARLIC ENZYME PRODUCING ALLICIN (ALLIINASE)- PROTEID OF PHOSPHOPYRIDOXAL

(Submitted by Academician A. I. Oparin, September 26, 1952)

B. P. Tokin (1) deserves credit for the discovery, in the paste obtained from the cloves of garlic, of the volatile antibacterial substance (phytoncide) and for its adaptation to clinical practice. Caballito and his collaborators (2) isolated the antibacterial active element of garlic in pure form and named it allicin. They established that this substance (with the characteristic odor of garlic) represents oxide of diallyldisulfide and is formed, by fermentation, from a more complex molecule on injuring the cloves of garlic.

The mechanism of the formation of allicin and the nature of its predecessor were explained by Stoll and Seebeck (3). These authors isolated from garlic a peculiar sulfur-containing aminoacid, (4) S-allyl-L-cysteinesulfoxide, which they named alliin. Under the influence of a specific enzyme, allinase, pulverizing the cloves or a garlic brings about a quick splitting up of alliin [coupled] with the formation of allicin, ammonia and pyroracemic acid; the transformation occurs as follows (3):

$$\underset{\text{alliin}}{CH_2{=}CH{-}CH_2{-}\overset{O}{\overset{\uparrow}{S}}{-}CH_2{-}CH(NH_2){-}COOH} \rightarrow$$

$$\rightarrow \underset{\text{allilsulfonic acid}}{CH_2{=}CH{-}CH_2{-}\overset{O}{S}H} + \underset{\text{a-aminoacrilic acid}}{CH_2{=}C(NH_2){-}COOH}; \qquad (1)$$

$$CH_2{=}C(NH_2){-}COOH \xrightarrow{+H_2O} NH_3 + CH_3 + CO{-}COOH; \qquad (2)$$

$$2CH_2{=}CH{-}CH_2{-}\overset{O}{S}H \xrightarrow{-H_2O} \underset{\text{allicin}}{CH_2{=}CH{-}CH_2{-}\overset{O}{\overset{\uparrow}{S}}{-}S{-}CH_2{-}CH{=}CH_2} \qquad (3)$$

Allinase catalyzes a reaction (1); transformations (2) and (3) proceed spontaneously at great speed.

Alliin is a B-replaced a-amino-acid with a sharply polar substitute in the B-position. [illegible] a-aminoacids with less sharp polar substitutes in the B-position, namely, tryptophane, cysteine, various thioethers of cysteine, serine and theronine, are subject to splitting in the overall scheme (4) in accordance with the above reactions (1+2) (6):

$$XCH_2\text{-}CH(NH_2)\text{-}COOH \rightarrow XH + CH_2{=}C(NH_2)\text{-}COOH \rightarrow$$

$$+H_2O \rightarrow NH_3 + CH_3\text{-}CO\text{-}COOH.$$

In recent years it has been established that enzymes accomplishing the indicated transformations namely, tryptophanaze (7), cysteinedesulfhydrase (8) B-thionase (9,10), Deaminases of serine (11,12) and threonine (12), are proteids of phosphopyridoxal.

A. E. Braunshtein and M. M. Shemiakin (6), who developed the general transformation theory of amino acids catalysed by pyridoxal enzymes, have pointed out the probable, pyridoxal-proteidic nature of alliinase.

In the present investigation we cite experimental evidence confirming this hypothesis. It appears probable that alliin is formed in the plant by the oxidation of S-allyl-L-cysteine (desoxyalliin) and that the latter is synthesized by the condensation of allylsulfide with serine (or, possibly, with cysteine), assisted by the phosphopyridoxal enzyme (see mechanism of the synthesis of cyctathionine (9) and other B-substituted a-aminoacids (6)).

EXPERIMENTAL PART

Experiments were conducted with the synthetic preparation of alliin containing besides the inherent (+)S-allyl-L-cysteinesulfoxide, its (-)S-diastereoisomer; we synthesized the preparation through oxidation with hydrogen peroide of L-desoxyalliin obtained from allylbromide and L-cysteine (4); both diastereoisomers are split by alliinase, with the splitting up of the (+)S-isomer proceeding at great speed.

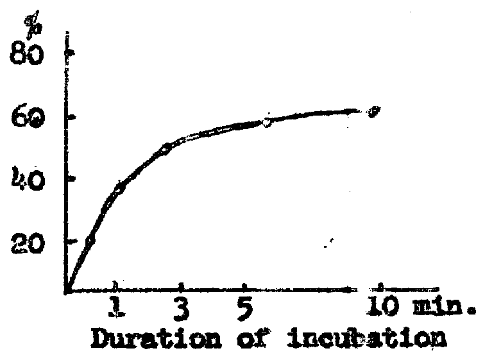

Fig. 1. Fission of alliin by alliinase

To prepare the solution of a partly purified alliinase (5) the cloves of garlic were quickly pulverized while being cooled in a mortar with quartz sand and 4 volumes of distilled water. The paste obtained was heated for 20 min. at 37° [C] and centrifuged. After the centrifuging, a 10% solution of CH_3COOH at pH 5.5 was added to the supernatant. The precipitate suspended in a phosphate buffer (M/15, pH 6.4) was used in experiments in the capacity of an alliinase preparation.

Certain experiments utilized partly purified extracts of alliinase inactivated ("apoenzyme" [apoferment]) by various means: 1) by dialysis against 0.01 M acetate buffer pH 5.0; 2) by ultraviolet radiation at a distance of 15 cm from a mercury quartz lamp for 60-90 min. and 3) by "aging" the enzyme through storage under toluene at 1-3° [C] (up to 14 days).

The activity of alliinase was tested as follows. Test specimens containing 0.5 ml [milliliter] of enzyme extract, 10 mg [milligram] of alliin and 2 ml of phosphate buffer (M/15, pH 6.4) common in a total volume of 5 ml were incubated for 10 min. at 37° [C]. In trichloracetic filtrates of the experimental specimens, the increment of ammonia was determined by the Convey-Byrne [Konvei-Birn] method and, in some experiments, the formation of pyroracemic acid, by the direct colorimetric determination of 2.4-dinitrophenylhydrazones of keto-acids (13)

Phosphopyridoxal (PP [FP]) was added in the form of pure Mg-salt (14) synthesized from pyridoxine by A. E. Braunshtein and R. M. Azarkh. Its actvity per unit weight is approximately 2-1/2 - 3 times higher than the activity of the preparation of PP [FP] Ba-salt utilized in previous work of our laboratory.

The splitting of the alliin preparation by alliinase, which we obtained in time is depicted graphically on fig. 1. The results cited show that the process of alliin splitting proceeds very rapidly; in 10 min. of incubation the decrease in the substrate reach 60-70%. Proceeding from this premise, all experimental specimens were incubated for 10 min.

Table 1.

Influence of Chemical agents upon the Activity of Alliinase.

Molar Concentration of poison	Hydroxylamine: Formation of N-NH3 in mg/g	Hydroxylamine: Inhibition in %	Phenylhydrazine: Formation N-NH3 in mg/g	Phenylhydrazine: Inhibition in %	Semicarbazide: Formation N-NH3 in mg/g	Semicarbazide: Inhibition in %
Control	3.39	--	3.39	--	3.39	--
2.10-3	0	100	0.10	97.2	0.42	87/6
10-3	0.16	95.2	0.19	94.4	0.42	87.6
10-4	0.16	95.2	2.42	28.7	2.06	39.3

As it is shown in table 1, the chemical agents obstructing the carboxyl group (hydroxylamine, phenylhydrazine and semicarbazide) inhibit the action of alliinase in concentration 10-3 M almost completely, with the greatest inhibition being caused by hydroxylamine.

In partly purified alliinase extracts the increment of ammonia nitrogen.

owing to the splitting of alliin, constitutes on the average 4.5 mg per gram of the initial garlic cloves (table 2). The numbers cited in table 2 show that the activity of alliinase decreases gradually in proportion to the length of storage at 1-3°C (for example, by 35-40% in 9 days and by 73% in 12 days), and, likewise, during dialysis against an acid buffer solution (by 42% in 20 hours and 75% in 48 hours.). Adding to such alliinase extracts ("apoenzymes") phosphopyridoxal in the amount of 20 y/5 ml reduces enzyme activity almost to its original level.

Table 2.

Activation by phosphopyridoxal of alliinase inactivated by means of "aging", dialysis or ultraviolet radiation (formation of $N\text{-}NH_3$ in mg per gram of garlic)

	Activity after storage			Activity after dialysis				Activity after ultraviolet radiation			
Initial Activity	Duration of storage in days	without additions	with PP/FP/ (20 y)	Initial activity	Duration of dialysis in hours	without additions	with PP /FP/ (20 y)	Initial activity	Duration of radiation in hours	without additions	with PP /FP/ (20 y)
4.20	7	3.06	4.40	4.20	8	4.07	--	4.60	1	0.37	[illegible].27
4.65	8	2.79	4.65	4.20	20	2.44	3.84	4.70	1	1.08	[illegible].39
4.40	9	3.46	4.40	3.60	26	1.34	3.19	4.70	1.5	0.30	[illegible].21
4.10	9	2.65	4.07	4.68	48	1.28	2.25				
4.65	12	1.24	3.12								

Ultraviolet radiation of a freshly purified alliinase solution for a period of 60-90 min. reduces considerably (by 70-90%) the cleavage of alliin. On adding PP [FP] to such extracts, the activity of alliinase becomes partly reduced without reaching the initial level. An analogous phenomenon was observed in experiments in which the alliinase extract used had been dialyzed for 48 hours or stored 12 days, which, obviously, can be explained by the partially destroyed albuminoid part of the enzyme.

In Fig. 2 a curve is shown demonstrating the dependency of alliin splitting on the amount of PP[FP] added to alliinase extract with a low activity (on the 9th day of storage at 1-3°[C]). The rates of alliin cleavage are shown in micromoles $N - NH_3$(1) and of pyroracemic acid (2) in the specimen; with a concentration of PP[FP] 5y/5 ml a maximum activity is reached.

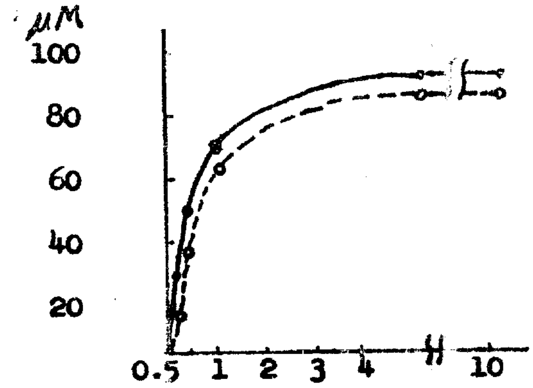

Fig. 2 Cleavage of alliin by alliinase (stored 9 days) in relation to the added amount of PP [FP]. 1--N--NH3; 2--keto acids (PP[FP]-- in γ per specimen of 5 ml dimensions; N--NH_3 and keto acid-- in μM per gr of garlic)

CONCLUSIONS. The alliinase of garlic possesses a high sensitivity to enzyme toxins inhibiting the carbonyl group. The activity of the "apoenzyme" of alliinase obtained through dialysis, "aging" or ultraviolet radiation is reduced on adding synthetic PP [FP]. These data show that the enzyme of garlic forming allicin is a proteid of phosphopyridoxal.

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REFERENCES CITED.

[1] B. P. Tokin, Phytoncides, M., 1948. [2]C. Cavzallito et al., Jour. Am. Chem. Soc., 66, 1952 (1844); 67, 1032 (1945). [3]A. Stoll, E. Seebeck, Adv. Enzymol., 11, 377 (1951). [5] A. Stoll, E. Seebeck, ibid, 32, 197 (1949). [6] A. Braunshtein, M. Shemiakin, DAN [Reports Academy of Sciences] 85, No. 5 (1952). [7] W. Wood et al., Jour. Biol. Chem., 170, 313 (1947). [8]A. Braunshtein, R. Azarkh, DAN, 71, 93 (1950); R. Azarkh, V. Gladkova, DAN, 85, No.1 (1952). [9]A. Braunshtein, E. Goriachenkova, DAN, 74, 429 (1950); E. Goriachenkova, DAN, 85, No. 3 (1952). [10]F. Blinkley et al., Jour. Biol. Chem., 194, 109 (1952). [11]C. Yanofsky, ibid., 194, 279 (1952). [12]J. Reissig, Arch. Biochem. Biophys., 36, 234 (1952). [13]A. Braunshtein, R. Azarkh, DAN, 85, No. 2 (1952). [14]M. Viscontini et al, Helv. Chim. Acta, 34, 1834, 2198 (1951)

D'iakova, G. A., [Phytoncides and plant diseases]. Uspekhi sovremennoi biologii. 35(2)1953 p: 257-270

The Soviet biologist B. P. Tokin discovered in 1928-1930 a property of higher plants of generating specific substances having a destructive effect on fungi, bacteria and protozoa.

Subsequent works by many scientists, established and theoretically generalized the vast distribution of such substances in nature among higher as well as lower plants. It was also established that these substances protect the plants which generate them against a number of microorganisms. The anti-microbal substances of higher plants were called phytoncides and the similar-in-action substances of lower plants - antibiotic. It has to be kept in mind that "phytoncides of any plant possess antibiotic properties, but by no means every antibiotic is a phytoncide, i.e. plays a protective role for the plant in its fight against microorganisms." (Tokin, 1951).

Thus, according to Tokin's definition, the phytoncides are a group of substances of plant origin, diverse in their chemical composition, but having one common property - that of being a protection in the control of fungi, bacteria, protozoa and other organisms.

Exhaustive information on phytoncides, their properties, chemistry, biological role in Nature, use in medicine, food industry, etc. is given in Tokin's works (1948, 1949, 1951).

Plants, during their life, are subject to many diseases, causal agents of which are fungi, bacteria and viruses.

B. P. Tokin suggested that there might be a connection between the generating of phytoncides by higher plants and the latter's immunity to certain diseases. The great Russian scientist I. I. Mechnikov looked for explanation of immunity, in the protective biological adjustments exisiting in live organisms. In plants the structural-anatomical, physiological and biochemical properties are those adjustments.

To protective chemical substances of plant belong: glucosides, phenol compounds, alkaloids, tannic substances, toxalbumins, organic acids etc. These substances generate as a responding reaction of the plant-host organism to the penetration of parasite into it.

The role of chemical substances in plant immunity is indicated, for example, by the following facts: Resistance of certain wheat varieties to rust (Puccinia triticina) is explained by the fact that they have a higher content of tannic substances than the non-resistant varieties.

Flax varieties resistant to fusariosis (Fusarium lini) have glucosides in their tissues - "liminarin." Extract from leaves and stems of flax, containing this glucoside, are toxic for the causual agent of the above disease.

E. Ya. Rokhlina established the connection between the resistance of the mustard family (Cruciferae) to Plasmodiophora brassicae and the content in the plants of glucosides of mustard oils.

The same author thinks that in the complicated complex of factors conditioning potato's resistance to Phytophthora, the chemical plant substances, in particular "solanin," play apparently a considerable role. In her experiments, substances extracted from Phytophthora - resistant plant varieties and containing the largest amount of "solonin," - inhibited the growth of Phytophthora's agents.

B. S. Drabkin (1951) indicates that the phytoncidal action of bird cherry leaves is connected with the content in their tissues of cyano-containing glucosides. One of the compoents of volatile fractions of bird cherry phytoncides is apparently hydrocyanic acid, which separates in hydrolysis of these glucosides.

However, B. A. Rubin and E. V. Artsikhovskaia (1948) emphasize, that "not the organic acids or glucosides, not sugar or bios, not peroxidase react to the penetration of parasite into the plant cell, but the entire living cell reacts as a whole." Therefor it is necessary to consider the immunity as: "an active physiological process; as areaction not of separate cell contents, but as a reaction of the entire living cell, the entire organism as a whole, in all the complex and manifold manifestations of its life activity."

B. P. Tokin (1948) defines plant immunity as "an entire complex of protective apparatis, connected with some structures and functions of organism, which may vary greatly in various stages of plant development and under various conditions of this development. This is a complex of occurrences in the relationships between the parasite and the host, which developed on a long road of evolution."

Around any plant, in the air, water, soil and finally, on the plant itself, there are enormous amounts of microorganisms. But only such species and forms, which adapted themselves to being parasites of the given plant, are capable to use the plant for their nutrition.

B. P. Tokin thinks that the faculty of higher plants as well as microorganisms to generate biologically active substances developed in the process of evolution of the plant world. This faculty of plants is their protective function and is to be understood in a wide biological sense, in which the direct bactericidal action of phytoncides is a particular case.

The action of phytoncides, like that of antibiotics, is specific and directed against definite species and groups of organisms. In case of weakening of the plant, due to some reason or other, it can not develop a sufficient amount of phytoncides for protection and is subject to an attack by parasites extrinsic until then.

In the opinion of scientists who work directly in the field of phytopathology, the role of phytoncides in generation of protective properties of plants consists in the following:

1. With their active effect on a number of microorganisms usually found on plant surface, the phytoncides prevent the transformation of saprophytic - for the given species - forms into parasitic.

2. Among microorganisms affecting the plant, there is a group of semi-parasites, which effect only weakened plants. The transition of semi-parasites to full parasites can take place when there is an insufficient amount of a complete lack of phytoncides in the plant.

However, it should not be assumed that only presence or absence of phytoncides determines the plant resistance to disease. Besides the fact that the production of phytoncides depends on the state of the plants.[1]) and this state is a function of the environment - the entire plant organism participates in the phenomenon of resistance (immunity) Gorlenko and Shneider, 1951).

2. Relation between phytoncides and the immunity of plants to certain diseases

The role of antibiotic substances in plant resistance to diseases, as well as the possibility of using these substances for control of plant diseases is of an undoubted theoretical and practical interest. However, this problem belongs to those which are not yet sufficiently studied, in spite of its interest and importance. Information in the literature in this field is very scarce. An attempt is made in this article to summarize the studies on relation between phytoncides and plant diseases.

A. T. Oparin and O. T. Kuplénskaia, studying in 1935 the resistance of the beet root during storing, established that the juice of the immune root inhibits the development of agents of the rot from storage mounds - fungi of Botrytis cinerea Phoma betae, etc.

The antibiotic action of the juice depended on the variety. Thus, juice of the immune Georgian beet inhibited the reproduction of yeast cells. The phytoncidal action of the juice is closely related to the general-physiological state of the root. Root juices of healthy beets of an immune variety inhibited to a considerable degree the development of yeast. Root juices with a distrubed resistance possessed a much lower fungicidal action. Drastic cooling off or heating of the root lowered the concentration of the antibiotic substance. The juice of the diseased root not only did not inhibit the reproduction of yeast, but even

1) Interesting data on the dependence of the degree of phytoncidal property of a plant on its state, are demonstrated in the works by P. A. Faloshentsev (1951). For purposes of diagnosis of healthy and weakened oak trees, they tested the phytoncidal properties of leaves and bast in the "slipper" of Paramaecium caudatum. It is established that the juice is highly protisticidal. The juices of older trees have higher phytoncidal properties than those of young and middle-aged trees. Each tree injury causes sharp increase in phytoncidal property of the juice. The The juice of the bast of dead trees is not phytoncidal.

increased it considerably.

Borzova (Tokin, 1948) indicates that the movement of zoospores of Phytophthora infestans in the juice from a tuber of a Phytophthora-resistant potato variety stops considerably sooner than in the juice from tubers of non-resistant varieties.

The zonal station of the All-Union Institute of Medicinal and Aromatic Plants discovered the antibiotic action of the extract of opium poppy flowers against a number of fungi causing disease of plants among them of the poppy itself (peronosporosis) (Kapustinskii, 1950).

A. F. Kapustinskii points out a very interesting circumstance. According to his data, the source for obtaining antibiotic extract is in the absolutely resistant pigmented forms of opium poppy. The coloring substance of these poppy forms is identified with the pigment belonging to the anthocyan group.

In G. F. Gauze's laboratory (1946) a strain of the ray fungus - the blue proactinomycete (Proactinomyces cyaneus) was isolated from the soil. The specific pigment - litmocidin - generated by this fungus, is an antibiotic possessing bactericidal properties. Its pigment group is built also similar to the anthocyan-pigment type, widely spread in the plant world.

In A. F. Kapustinskii's work (1950) Fedotov's data are given on the factor of anthocyan pigmentation of the bean which, on the basis of other factors, discloses a faculty to increase the resistance of peas to ascochytosis (Ascochyta pisi).

It is demonstrated in the same article that potato's immunity to canker is conditioned by the differentiation of varieties according to their anthocyan. Thus, of varieties with colored tubers only one - Berlikhingen, containing anthocyanin, is canker-resistant. At the same time other varieties (Early rose, Nero, Vol'tman), not containing anthocyanin, are not canker-resistant.

The facts given make it possible to establish the connection between the immunity of plants, bearers of anthocyanin and their antibiotic property towards pathogenic microbes.

An example of this kind of connection is also the antibiotic - tomatin. It is known that the juice of tomato varieties immune against wilt caused by Fusarium oxysporum f. lycopersici, inhibits the development of the agent of this disease (Lebedev, 1948). The antibiotic property of the juice is explained by its content of a substance which was called at first lycopersicin and then - tomatin. The seeds do not contain it, it forms in the plant 8 days after germination and reaches its highest concentration in the leaves of mature plants, lesser - in the root, minimum - in the stems and fruits.

Different susceptibility of various tomato varieties to wilt can be explained by the presence in them of tomatin. Susceptible varieties, even if they contain it under normal conditions, when affected, do not develop amounts sufficient for depression of agents. And resistant varieties have evidently the faculty, of accumulating, when affected, a necessary amount of tomatin, which prevents the spread of the disease.

The concentrate from tomato leaves is prepared by using 95% ethanol and after a series of operations it [tomatin] becomes a crystal substance.

Tomatin contains 57.5% of carbon, 8.32% of hydrogen, 1.35% of nitrogen. Its molecular weight is approximately 1050. The crystalline tomatin is soluble in ethanol, dioxane, methanol and propylene glycol; not soluble in water, ether, petroleum ether. The melting point is at 263-267°C. Tomatin depresses that growth not only of various Fusarium species, but of many Gram-positive and Gram-negative bacteria as well.

The problem of bactericidal properties of the plant juice is of great interest from the point of view of plant immunity.

The works by N. A. Krasil'nikov and A. T. Koreniako established that the tissues of stems, leaves and roots of healthy plants of clover, pea, kidney bean, vetch, alfalfa, lupine, vetchling - contained no bacteria. And, when bacteria are introduced artificially into the tissues, they perish relatively fast.

The action of the juice of these plants, as well as of cereals - corn, wheat and barley - were examined. The natural, undiluted juice of clover, sweet clover and alfalfa appeared to have the highest bactericidal property (bacteria perished after a few hours). Diluted juice (1:10, 1:20) had in the majority of cases, a bacteriostatic action and a greater dilution (1:50) stimulated growth.

Beside the nodule bacteria, the Azotbacter, Bact. herbicola. Ps. fluorescens, Bact. mycoides, Bac. subtilis, Bac. megatherium were also tested. Only two strains - Ps. fluorescens and Bac. subtilis developed in the juice of leguminous plants, the others did not develop or perished in 20-30 hours. The bactericidal action of stem, root and leaf juice was manifest in about equal degree. The cereal juices had a bacteriostatic action or none at all.

The bactericidal property of plant juice depended on conditions of their growth. Thus, the bactericidal property of the juice of plants grown under sterile condtions in pots or in hot-house flats was lower; than that of plants grown in a field.

L. E. Kramarenko's work (1949) is dedicated to the study of connection between the immunity of cotton varieties to gummosis (Bact. malvacearum) with the bactericidal property of cell sap. The following factors of cotton's resistance to gummosis were studied:

1) anatomical - the thickness of the cuticular layer of cotton leaves, size and distribution of stomata their number;

2) pH value of the cell sap;

3) presence of specific anti-bodies in the cell sap;

4) bactericidal property of the cell sap.

Study of all these resistance factors was carried out during various vegetation phases depending on plants affected and not affected by gummosis. It was established that one of the factors determining the varietal resistance of cotton to gummosis is the antibiotic action of its cell sap. The effect of cell sap is bacteriostatic towards Bact. malvacearum. And it was noted that there is a direct dependence between the bactericidal property of cell sap of cotton and the susceptibility of its varieties to gummosis. The higher the coefficient of bactericidal property of cell sap, the more resistant is the variety. The bactericidal power of the cell sap is a changing value, which increases with the plant's age. The lowest bactericidal property of the cell sap is observed during the cotyledon phase, the highest - during the budding phase.

In the laboratory of microbiology and bacterioses at the Institute of Zoology and Phytopathology, we (Kharkina, 1951) studied the comparative effects of garlic phytoncides on a series of phytopathogenic bacteria which represented the following groups:

1) causal agents of plant rots (Bact. phytophthorum, Bact. carotovorum);

2) yellow-pigmented plant parasites (Bact. malvacearum, Bact. heteroceum, Bact. vesicatorium);

3) Fluorescent plant parasites (Bact. citriputeale, Bact. atrofaciens, Bact. xantochlorum);

4) causal agents of plant canker (Bact. Tumefaciens).

It was established that the degree of effect of volatile as well as of non-volatile fractions of garlic phytoncides depends on species of phytopathogenic bacteria, their individual properties and amount of phytoncidal material. The most resistant to effects of garlic phytoncides was the group of agents of plant rots, the least - the group of the yellow-pigmented [parasites]; the intermediate position was taken by the group of fluorescent plant parasites.

This unequal sensitivity of phytopathogenic bacteria to phytoncides can be compared with the fact established by F. V. Khetagurova (1947) of biological differences among phytopathogenic bacteria. These differences are manifest in the adjustment of various phytopathogenic bacteria groups to existence on above-and-under-ground parts of plants. Thus, on green aboveground plant parts there are always representatives of the yellow-pigmented parasite group, and in the rhizosphere - of the white, putrid and fluorescent phytopathogenic bacteria.

F. V. Khetagurova's experiments (1950) established also that the "phytopathogenic bacteria populating the rhizosphere of the plants are more resistant to garlic and onion phytoncides as well as to antibiotics of fungal and bacterial origin. And bacteria on the surface of the green parenchyma of plants, i.e. the yellow-pigmented ones, manifest, on the contrary, a sensitivity both to antibiotics of lower organisms as well as to onion and garlic phytoncides."

This difference in the effects of garlic phytoncides depending on the species of phytopathogenic bacteria, has to be taken into consideration for practical use of garlic in the control of various agents of bacterioses. Garlic and onion are a powerful source of anti-microbial and anti-fungal elements. However, it is evident, that these plants can alsobe subject to bacterial and funal diseases.

V. G. Gramenitaskaia's (1949) and our own (1951) observations demonstrated that the primary cause for infection of garlic are bacteria preparing nutrient substrate for fungi of the Penicillium and Aspergillus type. The fungi do not spread beyond the garlic sections where the bacteria-affected areas are. Bacteria isolated from diseased garlic, according to their morphological, cultural biochemical properties and pathogenesis, are closely related to representatives of the group of agents of plant rots.

D. N. Teterevnikova-Babaian and S. A. Avakian (1950) discuss bacterial rot of onion seeds cuased by Bact. carotovorum.

Of the 10 phytopathogenic bacteria tested in our experiments, the highest effect was produced by volatile and non-volatile fractions of garlic phytoncides on Bact. citrputeale and the lowest - on bacteria isolated from the affected garlic. In Tu. T. Shneider's experiments the phytoncides of the Aurantiaceae did not affect the agents of the bacterial necrosis of Aurantiaceae (Bact. citriputeale) and at the same time they affected the bacteriosis agent of the fruit trees (Bact. cerasi) and of lilacs (Bact. syringae).

These facts indicate the adjustment of the given bacteria - which developed in the process of evolution - to a definite source of plant phytoncides as to an environment. They indicate also, that phytoncides of a plant are harmful only to microorganisms which are not adjusted to parasitic life on it.

3. Effect of phytoncides of higher Plants on phytopathogenic bacteria

K. T. Beltiukova and P. T. Kisel examined the effect of aqueous distillate of putsatilla herb (Anemona patens)[2] and "protoanemonin"[3] [?] obtained from this plant on a number of bacterioses agents.

Bactericidal and bacteriostatic effect of these substances is established for the following phytopathogenic bacteria" Bact. tobacum. Bact. coronafaciens, Bact. panici, Bact. medicaginis var. phaseolicola, Bact. phaseoli, Bact. andropogonis. The strength of the effect of antibiotic substances depended on the species of the agent, its individual strains, duration of exposure.

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2) There are indications in the literature on effects of juices of the following higher plants on phytopothogenic bacteria of kidney beans on Bact. carotovorum, Bact. amylovorum, of corn on Bact. stewarti and Bact. carotovorum, of cucumbers on Bact. carotovorum, of cabbage and wild mustard on Bact. compestre.

3) T. F. Rudakov (1951) discusses the content of volatile toxic substances in all species of clematis. The active part of clematis phytoncides is - "protoanemonin." Clematis phytoncides kill bacteria, fungi, protozoa and small rodents. V. T. Polianskii (1947) gives information on "khlozelin" [chloreillin?] - antibiotic substance of a fresh-water alga Chlorella vulgaris. This antibiotic is toxic not only for Gram-positive and Gram-negative bacteria, but for the organism which generates it as well, though in a certain concentration it can stimulate the reproduction of the alga - a very interesting fact, as an example of a relative expediency in Nature.

The tested effect of protoanemonin on germination and sprouting of seeds of oats, millet, tomato, kidney beans and cotton indicated their dissimilar sensitivity to this substance.[4] In connection with this fact, the use of "protoanemonin" as a possible agent for treatment of seeds, requires a thorough preliminary testing, for preservation of normal germination of seeds of each plant.

4. Effect of phytoncides of higher plants on phytopathogenic fungi

Borrzova observed the destruction of Phytophthora infestans spores exposed to 5 minutes of onion and garlic juices or 10 minutes of their vapors. Even the juice diluted to 1:2500 retained its antifungal action. She established also the effect of volatile phytoncides of onion leaves, skin and roots, garlic leaves and roots, fir needles, bird cherry buds and bark, and orange tree leaves on motile zoospores of the Phytophthora.

Additing of Toroptsev's "defenzonat" [ ] (chemically pure bactericidally acting onion element) diluted to 1:400,000, to the fluid containing zoospores of the Phytophthora, produced in 10 minutes a lysis of their walls (Tokin, 1948).

V. F. Kuprevich (1947) discusses the effect of fresh extracts from oats and potatoes on germination of uredospores Puccinia coronifera (crown rust of oats). In extracts from four oats varieties the percentage of spore germination was 15-45 and in extracts from four potato varieties - no growth was observed.

R. Vasudeva and B. Chona found that the spores of fungus-parasite of onion (Botrytis allii) and fungi-parasites of apple tree (Monilia fructigana, Fusarium coeruleim (lib)., Phytophthora erythroseptica and Phythium sp.), cannot germinate in the presence of juice of a foreign plant host. This can explain the resistance of the onion to the parasite of the apple tree and of the apple tree to the parasite of the onion.

Experiments on a series of plants carried out by T. Marchevskaia (1950), demonstrated that the horse-radish roots, edible roots of the black radish, juicy stems of aloe and geranium leaves, secrete phytoncides, which kill the spores of the Botrytis allii fungus (agent of the neet rot of onion) within 10 minutes.[5]

T. V. Michurin mentions his using of the bitter milky juice of the garden weed of lettuce (Lactuca scariola) for treatment of rust of roses (Phragmidium subcorticum). P. B. Turgenson (1952) rubbed 2-3 times a day the diseased sports of wild rose with onion, garlic juice. In two days the disease was liquidated.

Treatment of seeds

Agents of many plant diseases are transmitted by seeds. Therefore it is very important to obtain and store healthy seed material. It is achieved by selecting fruit seeds from unaffected plants and also by applying various methods of seed disinfection. The latter is carried out by treating the seeds with various chemical preparations, which procedure entails a series of shortcomings:

1) complete disinfection is not always achieved.

2) many of the most effective preparations represent various mercury compounds, very harmful for people who work with them.

5) G. T. Zaitsev (1951) describes the harmful effect, under laboratory conditions, of phytoncides of opening bird cherry buds on the culture of Penicillium and the house [domestic?] fungus Coniophora cerebella.

4) Pergova established in 1944 (Tokin 1948) the inhibiting action of onion and garlic phytoncides on cells of higher plants (water thyme, kidney bean, turnips, corn). An analyogous fact was discovered by T. T. Golubinskii (1949), when he studied the effect of phytoncides on germination of pollen grains. M. B. Rasumovich and S. M. Naumov (1951) established, that the most inhibiting effect on germination of rye and wheat seeds is produced by bird cherry, then garlic and onion.

The phytoncide discovery produced new effective treatment agents, the chief property of which is the safety for humans.

A. D. Lipetskaia (1946) established the positive action of volatile fractions of onion and horse radish phytoncides on spores of covered smut of barley Ustilago horde: 15 g. of paste of these plants were mixed with 100 g. of seeds. A 10 minute exposure was sufficient to cause a complete destruction of spores.

P. T. Korotkova also obtained positive results (1950) of effects of onion and garlic phytoncides on pea seeds affected by the Ascochyta pisa fungus. This is particularly important, since so far chemical substances were not found, which would disinfect the pea seeds sufficiently from the indicated fungus.

Raspopov, I. M., [Action of certain plant phytoncides on insects]. Priroda 1953(4):116 Apr. 1953.

During geo-botanical work in the Caucasian sanctuary, I carried out experiments for the study of effects of volatile fractions of phytoncides on flies and ants. As a basis for the study served the notions elaborated by B. P. Tokin in his monograph on toxic effects of volatile fractions of phytoncides of bird cherry leaves, on insects.

The experiments were carried out with plants in the vicinity of Krasnaia Poliana (Caucasus) and with plants from the park of the Caucasian sanctuary in Krasnaia Poliana. The leaves of the plants being tested were grated rapidly on a grater, the obtained paste--5-7 grams--was introduced in a 15cc. test tube; immediately after that several flies or ants were introduced into the tube. In experiments with coniferous species, finely cut needles were used (also in test tubes). Experimental plants were freshly cut.

29 plant species were studied; 25 of them were tree and shrub species. In seven cases very convincing results were obtained. The tulip tree, mountain ash, cherry laurel, juniper, Douglas fir, camphor laurel, Lawson's cypress, appeared to be toxic in various degrees for flies and ants; flies perished in 1-160 minutes, antis in 10-15 seconds.

In the Crimean National Sanctuary imeni V. V. Kuibysheva, the experiments on effects of volatile fractions of phytoncides on ants were continued.

The work was started with grass plants. The experimental procedure was similar. 10 different plant species were studied, but positive results were obtained only from large-flowered sage (Salvia grandiflora Ette).) Ants placed in the test tube with finely cut sage leaves, grown fin the valley of the Al"ma river, perished in 5-7 hours.

It should be mentioned that sage grown under unfavorable conditions, for example, on open slopes of the southern exposure of the Khyr-Alan range, had less phytoncide properties than sage plants from the Al"ma river valley at the foot of the Khyr-Alan range. Phytoncides of plants sown under unfavorable conditions killed the insects after 8-9 hours, or just "doped" them. Apparently they (phytoncides) have a narcotic effect.

Drabkin, B. S., [Action of benzoic aldehyde upon certain invertebrates]. Doklady Akademii Nauk SSSR 89(4):705-707. April 1, 1953.

We have cited evidence favoring the hypothesis that the phytoncidal action of the common birdcherry (Padus racemosa Lam.) is due to the presence of cyanogenic glucosides in its tissues, and that one of the components of the volatile fractions of birdcherry phytoncides, obviously, is prussic acid split off during hydrolysis of the above mentioned glucosides (1). This, however, does not mean that the volatile substances -- bearers of phytoncidal properties in birdcherry -- are being exhausted by prussic acid.

There are indications that a series of protozoa are comparatively insensitive to cyanides (2). Neither are enteric nematodes, which are capable of anoxybiotic metabolism, characterized by a high sensitivity to cyanides (3) . Yet, we have become convinced that the action of volatile phytoncides of birdcherry exert on these organisms is no less strong than the action they exert on rainworms which are oxybions [oksibliionty].

These observations encourage the hypothesis that the volatile fractions of birdcherry phytoncides represent a complex of substances which includes other components beside prussic acid. The direct relation observed between the phytoncidal action of birdberry on organisms comparatively resistant to cyanides and the isolation of prussic acid from birdberry may, possibly, be attributed to the circumstance that the formation of free hcn is accompanied by an equivalent isolation of another toxic element. In connection with this, other aglucones released in hydrolysis of cyanogenic glucosides deserve consideration.

It is known that glucosides of the amygdaline type prevalent in many representatives of the rose family and, particularly, in birdcherry become decomposed in the process of hydrolysis with the splitting off of two aglucones; prussic acid and benzoic aldehyde. As this occurs, a direct quantitative relationship exists between their formation.

To arrive at a solution of the question concerning the role of benzoic aldehyde which forms in the phytoncides of birdcherry, it was necessary to ascertain just how benzoic aldehyde affects the organisms of interest to us.

Literary data concerning the biological action of benzoic aldehyde are few and relate only to vertebrates, toxicity with respect to these was studied in conjunction with the use of benzoic aldehyde in the perfumery and food industries. There are indications that benzoic aldehyde is not poisonous if administered per os. V. I. Skvortsov (4) asserts that toxicity of pure benzaldehyde is minimal.

Data concerning the action of benzoic aldehyde on invertebrates we did not find. This fact prompted us to investigate the action of benzoic aldehyde upon some forms of the lower animals serving as objects of our experiments with phytoncides of birdcherry. The results obtained constitute the subject of the present report.

The work was conducted with a chemically pure benzoic aldehyde representing a liquid with the odor of bitter almond strongly reminiscent of the odor of crushed tissues of birdcherry.

A study was made of the action of benzaldehyde on Paramaecium caudatum, Euglena viridis, nematodes (Cosmocerca sp.) which act as parasites in the intestines of toads, on earthworms (Lumbricus sp.) and on flies (Musca domestica).

In the first series of experiments, the action of benzoic aldehyde fumes was investigated.

The action of benzaldehyde fumes upon Paramecium and Euglena was produced as follows. A certain amount of benzaldehyde (0.1, 0.2, and 0.3 ml [milliliter]) was placed at the bottom of a Petri dish. Over it, at a distance of 0.5 cm, was placed on cork supports, a slide with a drop of medium containing 15-20 Protista turned upside-down [obrashchennoi vniz]. Observations were conducted under a microscope through the lid of the Petri dish.

The action of benzoic aldehyde fumes upon nematodes was also studied, however, a drop of a physiological solution of sodium chloride containing two nematodes from the rectum of a toad was applied on the slide.

Experiments with rainworms were conducted in cans [biuky] of a 75 cm^3 holding capacity. Benzoic aldehyde was put on the bottom of the can and two rainworms were placed on a cardboard lattice resting on cork supports.

In the experiments with flies, a piece of cotton soaked in a fixed amount of benzaldehyde was put in a test tube, then 3-4 flies were quickly let into it, and thereupon the test tube was closed with a cork.

Control animals were placed under the same conditions as experimental ones, however, without benzoic aldehyde. The results obtained are cited on table 1

Table 1.

Action of benzoic aldehyde fumes upon some invertabrates (period of destruction since inception of experiments; average of 10 experiments)

Benzaldehyde entered in ml	Paramecia	Euglena	Nematodes	Rainworms	Flies
0.1	67 sec.	63 sec.	4.5 min.	11.5 min.	--
0.2	52 sec.	--	3.1 min.	9 min.	--
0.4	42 sec.	--	3 min.	8 min.	9 min.
Control	Alive	Alive	Alive	Alive	Alive

In the second series of experiments, the action of the solutions of benzoic aldehyde was studied. The solubility of benzaldehyde in water equals approximately 1:300. And so we used in the experiments benzoic aldehyde in a dilute of 1:300 or more. The aldehyde was diluted in a physiological solution of sodium chloride.

The experimental method used was the following: To a drop of medium containing 15-20 Paramecia or Euglena placed on the slide, was added a drop of a solution of benzoic aldehyde of a fixed concentration. The preparations were placed in damp compartments - Petri dishes lined on the inside with moistened filter paper.

In experiments with nematodes, two nematodes were placed in a drop of a physiological solution, then a drop of benzoic aldehyde solution was added. During the experiment the preparations were kept in a moist compartment. In control cases a drop of a physiological solution was added. In studying the action of benzoic aldehyde solutions on rainworms, the latter were put in a can containing 10 ml of benzoic aldehyde solution of an appropriate concentration (see table 2). Control worms were placed in a can with an equal amount of physiological solution.

Table 2.

Action of benzoic aldehyde solutions upon some invertebrates (period of destruction since inception of experiments; average of 10 experiments)

Initial delute of benzoic aldehyde added	Paramecia	Euglena	Nematodes	Rainworms
1:300	30 sec.	8.7 min.	59 min.	52 sec.
1:600	5.3 min.	17 min.	112 min.	10.7 min.
1:1200	18.2 min.	24 min.	Alive 3 hrs later	56 min.
Control	Alive	Alive	Alive	Alive

From the data on table 1 and 2 it is apparent that the solutions as well as the fumes of benzoic aldehyde exert a sharply toxic action upon the invertebrates which served as objects in our experiments.

The results obtained justify the hypothesis that benzoic aldehyde which forms in hydrolysis of certain glucosides may become a composite part of the phytoncide complex.

In the light of such a hypothesis one can visualize the biological importance of glucosides of the amygdalin type which accumulate in the tissues of a number of representatives of the rose family and, in particular, of the almond subfamily.

Being biologically neutral, these substances, upon injury of the plant easily hydrolyse and form two biologically very active aglucones; prussic acid and benzoic aldehyde capable of performing an essential role in the regulation of interspecific reciprocal relations and, particularly, in accomplishing protective functions.

Apparently, it is not only in the birdcherry, but also in other representatives of the rose family, particularly in such powerful phytoncide producers as cherry laurel (Laurocerasus officinalis), dwarf almond (Amygdalus-nana), the formation of phytoncides is associated with glucosides of the amygdalin type found in the tissues of these plants.

Of late, there has accumulated data (5) verifying the fact that other glucosides likewise have a share in the protective media of plants. Possibly this comprises one of the functions of glucosides whose physiological role in the plant organism cannot, until recently be considered as conclusively established.

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